SUMMARY OF THE INVENTION

It is an object of the present invention to combine the use of liposomes in an immunoanalysis method with a flow injection analysis system.

It is a further object of the present invention to prepare liposomes as immunoreactants by noncovalent coupling of biotinylated binding agent with biotinylated liposomal lipids through the protein, avidin.

It is a further object of the present invention to covalently immobilize antibody fragments in a special orientation to develop an accurate and efficient immunoanalysis method.

It is yet a further object of the present invention to combine automated sampling and analysis with reusable immuno-reactants.

In one aspect, the present invention is directed to a method of immunoanalysis which combines immobilized immunochemistry with the technique of flow injection analysis, and employs microscopic liposomes as carriers of detectable reagents. Liposomes are modified on their surface with analytical reagents, and carry in their internal volume a large number of detectable marker molecules. The modified liposomes can bind to antibody fragments immobilized on an immunoreactor column. Liposomes left unbound, due to the presence of an analyte in a competitive assay, can be lysed and the marker component concentration detected. This results in a quantification of the concentration of the analyte. Non-competitive assays can also be performed using the liposomes.

Another embodiment of the present invention relates to binding assays involving modified liposomes whose marker compound can be quantified at a later step in the assay directly, or by disrupting or lysing the liposomes. The amount of the marker compound released can then be related to the concentration of analyte in the sample being tested. Similar assays may be performed manually, without the use of flow injection analysis and are contemplated as equivalents within the scope of this invention.

The present invention also relates to a liposome immunoassay which involves incorporating fluorophores into liposomes, and modifying the liposome membrane with an immunoreactive reagent. The present invention also covers another use for liposomes in a homogeneous assay wherein liposomes are noncovalently derivatized using methods generic to all immunoreactive reagents.

In another embodiment of the present invention, the intensity of scattered light is quantitated as a measure of liposome aggregation in response to a concentration-dependent immunospecific reaction. In this embodiment, encapsulation of marker molecules into liposomes is not required.

The present invention also relates to assays for the purpose of diagnostics and rapid detection and quantitation. The simplicity of the techniques, and the fact that they are relatively rapid, make them very applicable to low-technology environments, which may include physician's offices, or walk-in clinics. For the same reasons, it will have application to field use, such as for testing of water purity.

The invention may be more fully understood with reference to the accompanying drawings and the following description of the embodiments both discussed herein and shown in those drawings. The invention should be recognized as contemplating all modifications within the skill of an ordinary artisan.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** illustrates a method of immobilizing the antigen binding portion of antibodies;

FIG. **2** is a schematic representation of the solid-state immunoliposome assay of the present invention;

FIG. **3** is a schematic diagram of a flow injection immunoanalysis system of the present invention;

FIG. **4** is a schematic representation of flow injection analysis assays of the present invention;

FIG. **5** is a schematic diagram showing derivatization of liposomes prepared for use in the present invention;

FIG. **6** is a schematic diagram comparing the steps of an enzyme-linked immunosorbant assay to an immunosorbant assay of the present invention;

FIG. **7** is a graph which shows a comparison between the relationship of relative fluorescence intensity and the log of theophylline concentration of an immunosorbant assay of the present invention and an enzyme-linked immunosorbant assay;

FIG. **8** is a graph showing the immunospecific aggregation of liposomes in relation to time; and

FIG. **9** is a graph showing the relative scatter intensity of light in relation to the concentration of mouse IgG when tested in accordance with an embodiment of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

Immobilization of antibodies which bind an analyte of interest in a FIIA reactor is the first step in developing a reusable, automated immunoanalyzer. Noncovalent association of antibodies with glass or plastic surfaces is the immobilization technique commonly used for many ELISA and RIA assays. In the process of binding during assays of this type, many potential antibody binding sites are blocked, activity is lost, and precise quantification of number and affinity of remaining sites is not known, resulting in a concomitant loss of analytical accuracy. The most frequently used chemistry for covalent immobilization is through amine groups of the antibody protein, of which there are many. The random selection of reactive groups leads again to loss of activity when amines near the binding site are involved.

In one embodiment, phospholipid molecules derivatized with antigen are inserted into the membrane of each liposome. The modified liposomes can compete with analyte molecules in a sample for binding to immobilized antibody fragments on an immunoreactor column. Liposomes are selected which will not disrupt upon binding to a solid phase support, e.g., upon binding between a binding agent incorporated into the surface membrane of a liposome and a receptor on a solid phase support. As a result, for every liposome not bound to the column, 1×10^5 molecules of marker compound are available for detection and quantification. Non-competitive assays can also be performed using derivatized liposomes and, inter alia, sandwich-type assay schemes. Automated sampling and analysis can be combined with reusable immunoreactants when regeneration of the immunoreactor is performed. The invention is also directed to products useful in said assay, especially in kit form.

According to an important embodiment of the present invention, the antibody **18** is cleaved by the enzyme pepsin, and the disulfide bridge **19** at the "hinge" region of the resulting fragment is then chemically reduced to sulfhydryl groups. The antigen-binding portion **20** of